

# Comparative Spermatology of Gastrotricha

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## ABSTRACT

Sperm morphology of nine gastrotrich species belonging to the two orders Macrotrichida and Chaetonotida has been examined. By comparison with the limited data in the literature it has been possible to determine a sperm model for macrotrichids: long, corkscrew-shaped acrosome often showing two different portions and containing a striated tube; spring-shaped nucleus surrounding the mitochondria; tail with an axoneme surrounded by a striated cylinder. Within Chaetonotida the spermatozoa are highly diverse: in the family Xenotrichulidae they are characterized by an uncondensed nucleus, two extremely long paraacrosomal bodies, a single mitochondrion and accessory fibres in the tail. The other chaetonotid families show extremely atypical spermatozoa, formed by simple rods of chromatin. There are no evident characters in common among the spermatozoa belonging to the two orders, nor similarities with other aschelminth sperm models.

## RÉSUMÉ

### Spermatologie comparée des Gastrotricha

La morphologie du spermatozoïde a été étudiée dans neuf espèces de Gastrotriches appartenant aux deux ordres Macrotrichida et Chaetonotida. Grâce à une comparaison avec les données limitées de la littérature, il a été possible de définir un modèle spermatique pour les Macrotrichida: long, avec un acrosome en tire-bouchon qui présente souvent deux portions et contient un tube strié, un noyau hélicoïdal entourant la mitochondrie, une queue avec un axonème entouré par un cylindre strié. Chez les Chaetonotida le spermatozoïde est très diversifié: dans la famille Xenotrichulidae, il est caractérisé par un noyau non condensé, deux corps para-acrosomaux extrêmement longs, une mitochondrie unique et des fibres accessoires dans la queue. Les autres familles de Chaetonotida montrent des spermatozoïdes très atypiques, formés par de simples baguettes de chromatine. Il n'y a pas de caractères spermatologiques en commun unissant les deux ordres, ni de similarités avec les modèles spermatiques des autres Aschelminthes.

Gastrotricha are small (80  $\mu$ m - 1.5 mm) pseudocoelomate animals living in the sediments of aquatic environments. The order Macrotrichida comprises about 230 species exclusive of marine and brackish interstitial environments, whereas the order Chaetonotida, with about 320 species, is mainly freshwater dwelling, both in interstitial and epibenthic habitats. The two orders have different reproductive modes: Macrotrichida are all amphimictic and hermaphrodite, whereas Chaetonotida are thought to be mainly parthenogenetic with the exception of three hermaphrodite, marine genera [3].

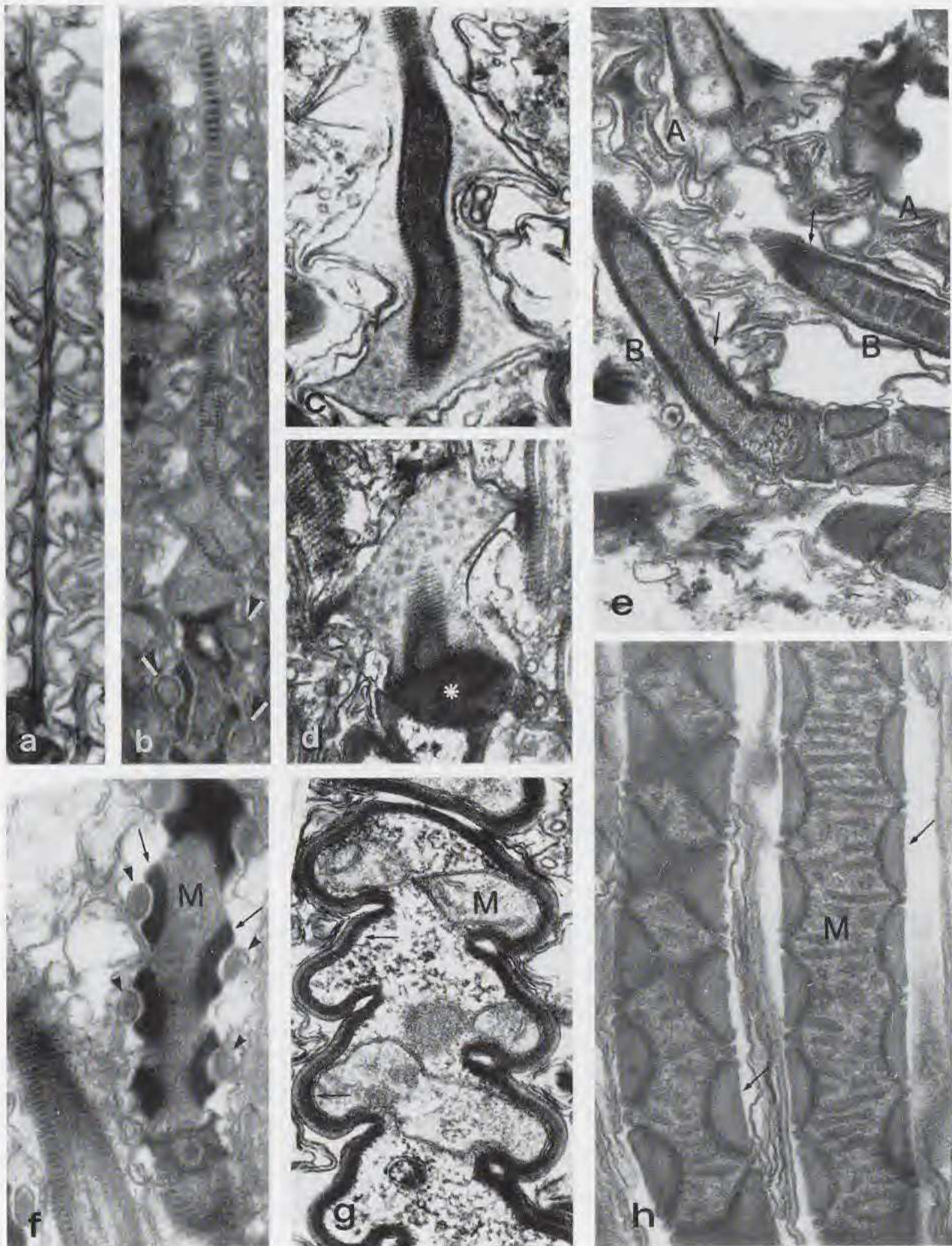
TABLE 1. — List of gastrotrich species included in the present review and relevant literature references.

Order	Family	Species	Reference
Macrodasysida	Macrodasysidae	<i>Macrodasys</i> sp.	[10]
	Lepidodasyidae	<i>Mesodasys laticaudatus</i>	[6]
		<i>Cephalodasys maximus</i>	[8]
		<i>Pseudostomella etrusca</i>	Present study
	Thaumastodermatidae	<i>Tetranchyroderma</i> sp. 1	Present study
		<i>Tetranchyroderma</i> sp. 2	Present study
		<i>Tetranchyroderma papii</i>	Present study
		<i>Diplodasys ankei</i>	Present study
		<i>Turbanella ambronensis</i>	Present study
		<i>Turbanella cornuta</i>	[12, 13]
Chaetonotida	Neodasyidae	<i>Neodasys</i> sp.	[11]
	Chaetonotidae	<i>Chaetonotus maximus</i>	[3]
		<i>Lepidodermella squamata</i>	[9]
		<i>Heteroxenotrichula squamosa</i>	[7, Present study]
	Xenotrichulidae	<i>Xenotrichula intermedia</i>	[7, Present study]
		<i>Xenotrichula punctata</i>	[7, Present study]

Gastrotrich spermatozoa are only imperfectly known (Table 1). In fact only two sperm models of Macrodasysida have been completely described [6, 12, 13], and among Chaetonotida we know with some details only the extremely atypical, perhaps relictual spermatozoon of *Lepidodermella squamata* [9]. Recently some data on the xenotrichulid spermatozoa have been reported [7]. Data on other species are scattered, often restricted to single micrographs, and in some cases inconsistent even within single families. With this poor knowledge, delineation of a generalized sperm model for Gastrotricha is extremely difficult.

In the order Macrodasysida the spermatozoa are filiform cells comprising a sequence of acrosome, nucleus and tail, and devoid of any recognizable midpiece (Fig. 3). Both acrosome and nucleus are corkscrew-shaped: the acrosome shows different regions with complex differentiations [6, 10, 12, 13]; the nucleus is spring-shaped in the lepidodasyid *Mesodasys laticaudatus* [6] and in the turbanellid *Turbanella cornuta* [12, 13], the nuclear spring surrounding the mitochondria in both species. In the lepidodasyid *Cephalodasys maximus*, the nucleus is

FIG. 1. — Ultrastructural features of macrodasysid gastrotrich spermatozoa. **a:** *Tetranchyroderma* sp. 1: anterior part of an acrosome with the striated tube. x 21 000; **b:** *Diplodasys ankei*: base of the acrosome with the wider portion of the striated tube. A further tubular structure possibly continuous with the acrosome coils around the nucleus (arrowheads). x 40 000; **c, d:** *Pseudostomella etrusca*: main (c) and basal (d) portion of the acrosome: the striated tubule ends in a dense material (asterisk). x 40 000; **e:** *Turbanella ambronensis*: anterior (A) and basal (B) part of the acrosome; the obliquely striated sheath surrounding the basal portion of the acrosome is visible in grazing sections (arrows). x 45 000; **f:** *Diplodasys ankei*: basal part of the nucleus (arrows) surrounding the mitochondrion (M) and involved by the coiled tubular structure (arrowheads). Note the curious structure at the base of the flagellum (compare with Fig. 2a). x 40 000; **g:** *Pseudostomella etrusca* nuclear region: in this species the chromatin is reduced to a thin lamina (arrows) involving the cytoplasm with some mitochondria (M). x 40 000; **h:** *Turbanella ambronensis*: the nucleus (arrow) is spring-shaped and surrounds a single, long mitochondrion (M). x 60 000.



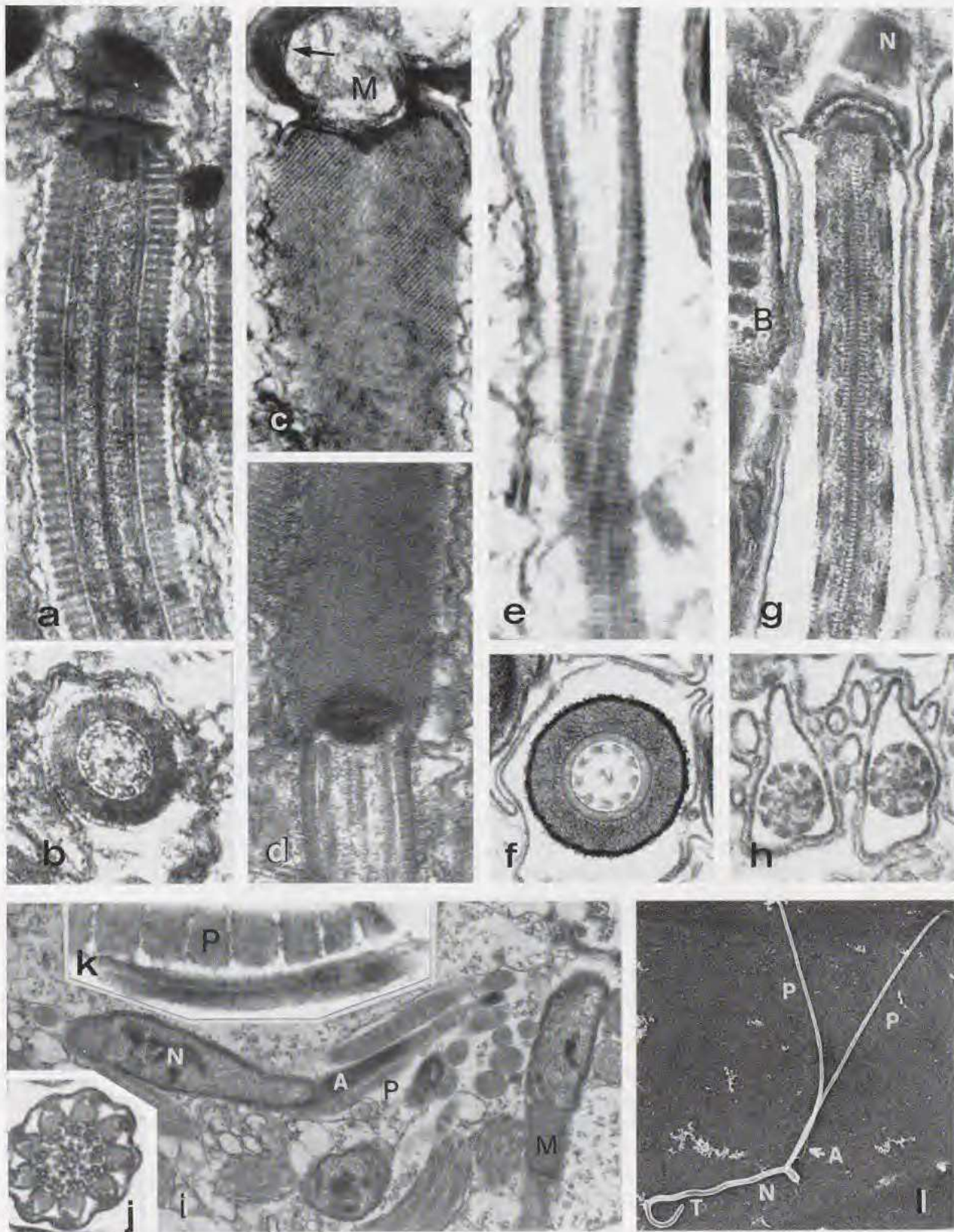
twisted in the apical portion and spring-shaped, containing the single mitochondrion only in the basal portion [8]. In contrast, in the macrodasyid *Macrodasys* sp. [10] the nucleus is straight and surrounded by a mitochondrial helix involving also most of the acrosome. A similar situation was reported also in *Thaumastoderma* sp. [11].

The tail has a 9+2 axoneme enclosed in a striated cylinder in all the macrodasyid species studied so far with the sole exception of *Turbanella cornuta* [12, 13] and, perhaps, *Acanthodasys* sp. [11] (however, the only micrograph published refers to spermatids).

For the order Chaetonotida we only have a single published micrograph of the spermatozoon of the interesting genus *Neodasys* from the monogeneric family Neodasyidae [11]: it shows a very simple and undifferentiated conical acrosome followed by a nucleus perhaps surrounded by a mitochondrial helix. Of the three genera composing the family Xenotrichulidae, *Draculiciteria* is parthenogenetic, whereas *Xenotrichula* and *Heteroxenotrichula* have very peculiar spermatozoa [7] with a small and simple acrosome followed by an uncondensed rectilinear nucleus, a single mitochondrion and a tail with nine peripheral accessory fibres. Furthermore, two of the xenotrichulid species show extremely long paraacrosomal bodies of unknown function. All the other chaetonotid families, comprising hundreds of species, are parthenogenetic. However, hermaphroditic individuals of most genera have been discovered both in culture and in nature and production of the spermatozoa during the long post-parthenogenetic phase of the life cycle has been demonstrated in at least four genera of the family Chaetonotidae [3, 9]. Low numbers of these spermatozoa are usually present, with four different morphologies. In *Lepidodermella squamata* [9] and *Chaetonotus maximus* [3] the spermatozoa are only rods of condensed chromatin surrounded by plasma membrane without organelles of any kind.

Thus, comparative spermatology of Gastrotricha is still in its infancy. For many years we have undertaken a study of selected gastrotrich species to extend our knowledge of their spermatozoa. However, the technical problems in working with such material (extremely small size of these animals; difficult fixation; problems with embedding and orientation of specimens within the resin; failure of attempts to rear macrodasyids in the laboratory) have impeded our project. Knowing the importance of the spermatozoa as systematic characters in comparisons within and among taxa, in this paper we will present some work in progress on previously undescribed gastrotrich species. We will focus our attention on the family Thaumastodermatidae, with *Pseudostomella etrusca*, three species of *Tetranchyroderma*, and *Diplodasys ankei*; we will describe a previously unknown spermatozoon from the Turbanellidae, *Turbanella ambronensis*; finally we will extend the description of the sperm of the three Xenotrichulidae [7]. We will present some ultrastructural details of the species described and three-dimensional reconstructions of four gastrotrich spermatozoa.

FIG. 2. — Ultrastructure of the spermatozoa of gastrotrichs. **a-h**: macrodasyids; **i-l**: chaetonotids. **a, b**: *Diplodasys ankei*: longitudinal (a) and cross (b) section of a tail to show the structure of the striated cylinder. x 60 000; **c, d**: *Pseudostomella etrusca*: longitudinal sections of the crystalline structure situated between the nucleus (arrow) and the tail. M, mitochondrion. x 60 000. **e**: *Tetranchyroderma* sp. 1: longitudinal, sagittal (top) and tangential (bottom) section of the tail showing the structure of the striated cylinder. x 60 000; **f**: *Tetranchyroderma* sp. 2: cross section of a tail showing the double striated cylinder. x 60 000; **g, h**: *Turbanella ambronensis*: longitudinal (g) and cross (h) sections of a tail showing no striated cylinder. N, nucleus; B, basal portion of the acrosome. x 60 000; **i, j, k**: *Xenotrichula punctata*; **i**: low power view of some spermatozoa: N, nucleus; A, acrosome; P, paraacrosomal body; M, mitochondria. x 20 000; **j**: cross section of a tail. Note the prominent accessory fibres. x 60 000; **k**: longitudinal section of an acrosome (bottom) and a paraacrosomal body (P). x 60 000; **l**: *Heteroxenotrichula squamosa*: S.E.M. view of a whole spermatozoon. P, paraacrosomal body; A, acrosome; N, nucleus; T, tail. x 2 800.



## MATERIAL AND METHODS

Gastrotrichs were extracted from sandy sediments collected in some localities of the Tyrrhenian and Adriatic coasts of Italy by decantation and narcotization with 7% MgCl<sub>2</sub>. Observations on living specimens were carried out by means of a Leitz Dialux microscope equipped with Nomarski optics and phase contrast. Moving spermatozoa within the testes, and after isolation, were recorded on videotape.

**Transmission Electron Microscopy (TEM).** Specimens were fixed in a 0.1 M phosphate buffered (pH 7.3) mixture of paraformaldehyde, glutaraldehyde and picric acid with addition of sucrose (SPAF) [5], postfixed in 2% aqueous osmium tetroxide, washed in 0.1 M cacodylate buffer, dehydrated in a graded acetone series, pre-stained *en bloc* with uranyl acetate in 70% acetone and embedded in araldite. Sections were cut with an Ultratome Nova LKB, triple stained following DADDOW [4], carbon coated and observed under a JEOL 100 XS electron microscope.

**Scanning Electron Microscopy (SEM).** The spermatozoa were isolated from living animals and fixed with the same mixture used for TEM, dehydrated in a graded ethanol series, critical point dried with CO<sub>2</sub>, coated with gold-palladium and observed with a Philips XL40 or a Cambridge Stereoscan 250Mk2.

## OBSERVATIONS

*Macrodasyida*

**Family Thaumastodermatidae.** The spermatozoa of *Pseudostomella etrusca* (Fig. 4) show a complex, corkscrew-shaped acrosome 5-7 µm long containing a matrix in which a tubular striated structure is immersed (Fig. 1c, d). The striated tube ends basally in an area of dense material which forms the base of the acrosome (Fig. 1d). The nucleus follows with the typical shape of a hollow corkscrew (Fig. 1g), about 20 µm long. The chromatin is visible as a 0.1 µm thick external sheath whereas the inner cavity contains some cytoplasm with numerous mitochondria (Figs 1g, 2c). Between the nucleus and the tail there is a crystalline structure, about 1.6 µm long and 0.5 µm in diameter (periodicity of about 13 nm) (Fig. 2c, d). The tail consists of a 9+2 axoneme surrounded by a striated cylinder formed by a pile of rings about 13 nm thick connected by thin threads (Fig. 2d).

We have examined three species of the genus *Tetranchyroderma*: an unidentified species from Tyrrhenian sea (*T. sp. 1*); an undescribed species from the Maldive archipelago (*T. sp. 2*); and *T. papii* from the Tyrrhenian sea. The acrosome of *T. sp. 1* and *T. papii* show an apical filament followed by a corkscrew region for a total length of approximately 8 µm (*T. papii*). The acrosome axis is a striated tube (Fig. 1a) ending in a basal mass of dense material touching the nucleus (*T. sp. 1*). The ribbon-like nucleus coils around the single long mitochondrion in *T. sp. 1*. An additional tubular structure runs for two gyres around the nuclear apex in *T. sp. 1* whereas it wraps around the whole nucleus in *T. papii*. The tubular structure continues the pitch of the acrosome, and may also be structurally continuous with it. A crystalline structure similar to the one already described in *P. etrusca* is visible in *T. papii* and in *T. sp. 2*, but is absent in *T. sp. 1*. The tail axis is a 9+2 axoneme surrounded by a striated cylinder formed by a pile of disks 15 nm thick connected by thin threads (*T. sp. 1* and *T. sp. 2*) (Fig. 2e). In *T. sp. 2* a second external layer is present, similar in texture to the crystalline structure at the base of the tail. This additional sheath is thicker (up to 120 nm) at the base of the flagellum (Fig. 2f), then flattens to a size comparable to that of the striated cylinder. Images of "empty" tails, in which the striated cylinder is present but there is no axoneme, suggest that the axoneme is shorter than the striated cylinder.

*Diplodasys ankeli* has a long, thin, corkscrew-shaped acrosome (Fig. 1b) in which two regions can be recognized: the anterior one, 0.15 µm in diameter with a helical pitch of about 0.26 µm contains a twisted column with alternate dark and pale cross bands, whereas the basal portion, about 1 µm long, contains a twisted striated tube. The following nucleus is a spring of condensed chromatin surrounding the single mitochondrion (Fig. 1b, f). A tubular structure possibly continuous with the acrosome coils around the nucleus for its whole length (Fig. 1b, f). The tail has a 9+2 axoneme enclosed by a striated cylinder formed by a pile of discs (Fig. 2a, b).

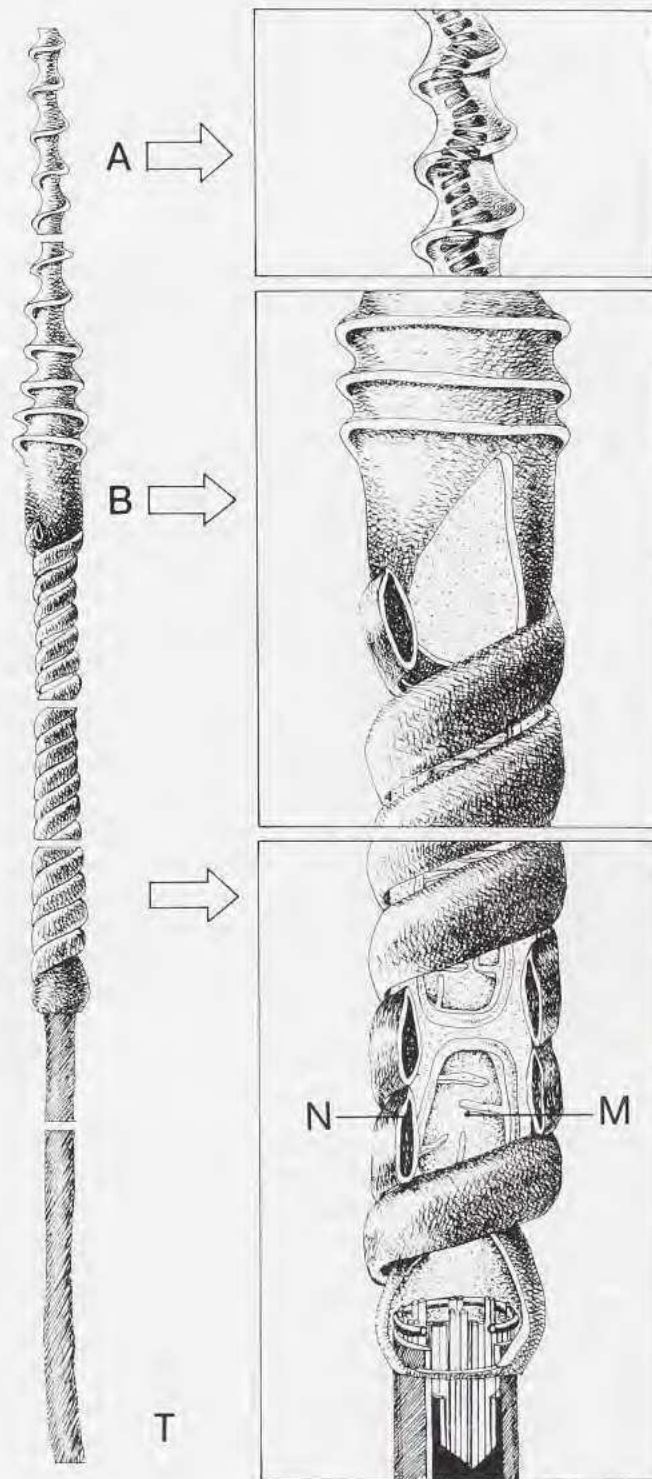


FIG. 3. — Schematic drawing of *Mesodasys laticaudatus* spermatozoon. A, anterior portion of the acrosome: the striated column is visible in the detail (right); B, basal portion of the acrosome. A detail is visible at right; N, nucleus; M, mitochondria; T, tail.

*Family Turbanellidae.* The acrosome of *Turbanella ambronensis* has two different regions (Figs 1e, 5): the anterior one is thin (0.2  $\mu\text{m}$ ), corkscrew-shaped and contains a twisted axis made of alternating dense and transparent bands; the basal portion is long and rectilinear and shows an obliquely striated sheath surrounding the acrosome content (Fig. 1e). This is formed by a pile of thick (60-80 nm) disks immersed in a transparent matrix. The nucleus is ribbon-like, D-shaped in cross section (Fig. 1h), and coiled to form a spring inside which a single, long mitochondrion is located. The tail has a normal 9+2 axoneme but no striated cylinder (Fig. 2g, h).

### *Chaetonotida*

*Family Xenotrichulidae.* We have studied the spermatozoa of three xenotrichulid species: *Heteroxenotrichula squamosa*, *Xenotrichula intermedia*, and *X. punctata*. The spermatozoa of these species have many characters in common, thus they will be described together. The xenotrichulid spermatozoa are characterized by a sequence of acrosome, nucleus, mitochondrion and tail (Fig. 6). The acrosome is a small structure (3.8  $\mu\text{m}$  long with a diameter of 0.1  $\mu\text{m}$  in *H. squamosa* and 2.9  $\mu\text{m}$  long in *X. punctata*) containing an acrosome vesicle and a large amount of periacrosomal material (Fig. 2k, i, l). Two projecting structures parallel to the acrosome are inserted at the anterior extremity of the nuclear region in *X. punctata* and *H. squamosa* (Fig. 2i, l) where they are 20  $\mu\text{m}$  long with a diameter of 0.2  $\mu\text{m}$ . These structures are not surrounded by a plasma membrane and consist of electron dense disks 0.1  $\mu\text{m}$  thick connected by thin threads (Fig. 2k); we have called them paraacrosomal bodies [7]. In the insertion area the plasma membrane of the spermatozoon is uninterrupted [7], suggesting that the paraacrosomal bodies are external to the cell. *X. intermedia* is an exception in having no acrosome nor paracrosomal bodies. The nucleus is characterized by a scarcely condensed chromatin in the three species examined. Only in *X. punctata* scattered dense globules of condensed chromatin were observed (Fig. 2i), whereas in the other species examined the chromatin resembles that of a somatic cell. A single, conventional mitochondrion of variable length follows the nucleus (Figs 2i, 6). A short tail is the last portion of the xenotrichulid spermatozoon. In its principal tract the axoneme is surrounded by nine large electron dense accessory fibres which are external to and in correspondence with each axonemal doublet (Fig. 2j). They have a somewhat striated appearance in grazing longitudinal sections whereas in cross section they appear pear-shaped with the thinner extremity directed towards the cell membrane and the larger one towards the doublets. The last portion of the *X. intermedia* spermatozoon shows a short tract of the axoneme devoid of accessory fibres.

## DISCUSSION

### *Macrodasysida*

The spermatozoa of members of four macrodasysid families have been examined up to now: Lepidodasyidae, Macrodasysidae, Thaumastodermatidae, and Turbanellidae (Table 1). The spermatozoa of Lepidodasyidae, Thaumastodermatidae (with the reported exception of *Thaumastoderma* [11]) and Turbanellidae have a common general architecture: the acrosome is at least in part corkscrew-shaped and contains various materials with different aspects; the nucleus is always, at least in part, spring-shaped and contains some cytoplasm and one or more mitochondria; a true basal body is lacking. Interspecific variations affect the acrosome which may be divided in two portions, the anterior one containing a twisted axis made of dense and pale disks, as in *Mesodasys laticaudatus* [6], *D. ankei* and *T. ambronensis*. The basal portion of the acrosome may be rectilinear (*T. ambronensis*, *M. laticaudatus*) and contain a hollow striated tube, as in *D. ankei* and *Cephalodasys maximus* [8]. A twisted hollow striated tube may run for the whole length of the acrosome, as in *P. etrusca* and *T. sp.1* where a mass of dense material is also present at the base of the acrosome. *Cephalodasys maximus* has an apical acrosome vesicle protruding anteriorly [8]. If this report were confirmed, the real nature of the long structure

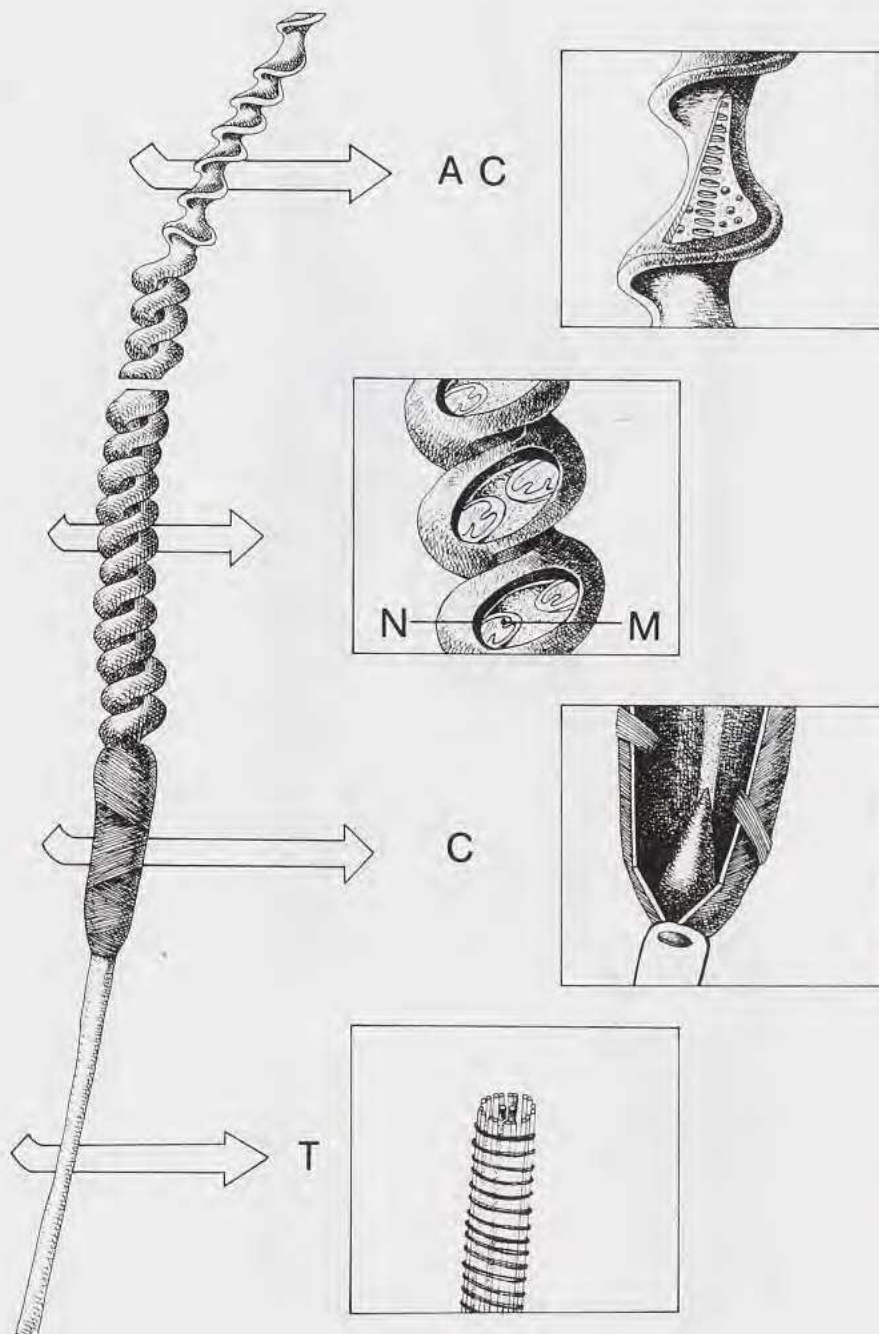


FIG. 4. — Schematic drawing of *Pseudostomella etrusca* spermatozoon. AC, acrosome: in the enlarged detail (right) the inner structure of the acrosome is visible; N, nucleus; M, mitochondria; C, crystalline structure; T, tail.

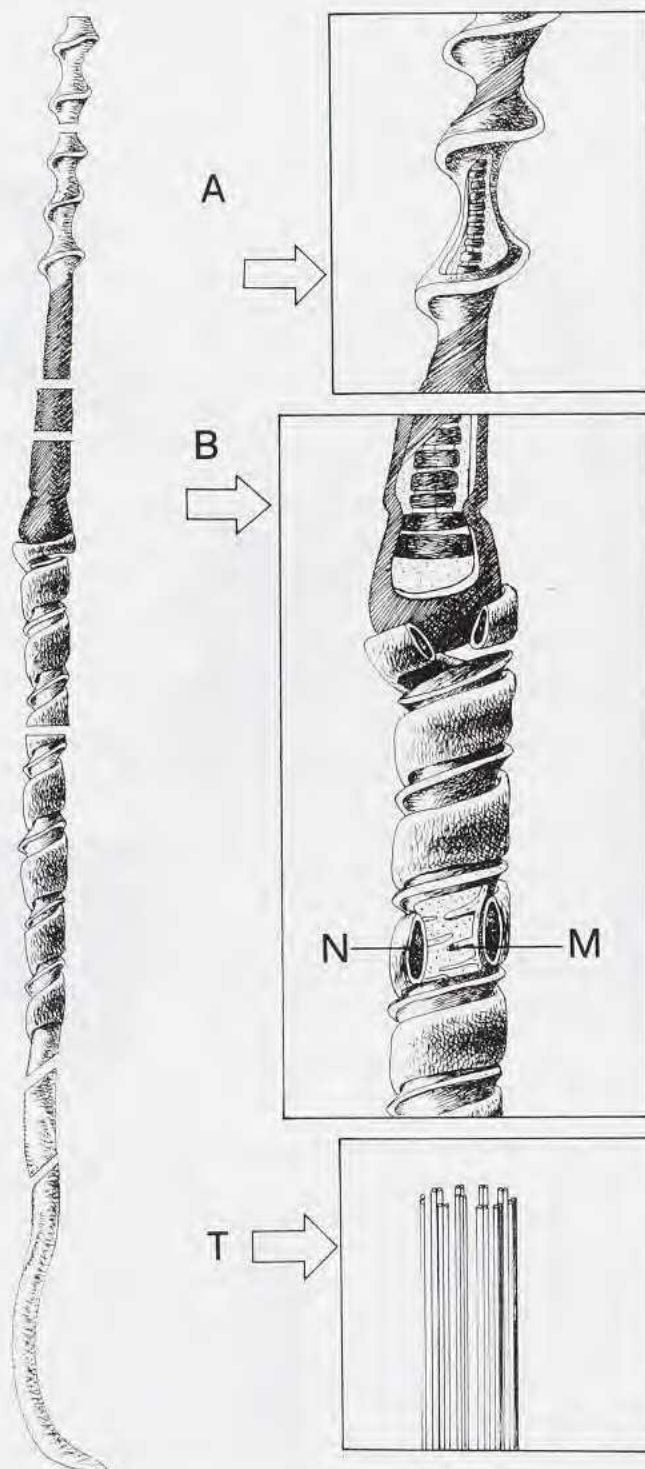


FIG. 5. — Schematic drawing of *Turbanella ambronensis* spermatozoon. A, anterior portion of the acrosome with the striated column inside (detail at right); B, basal portion of the acrosome with the obliquely striated sheath; N, nucleus; M, mitochondria; T, tail.

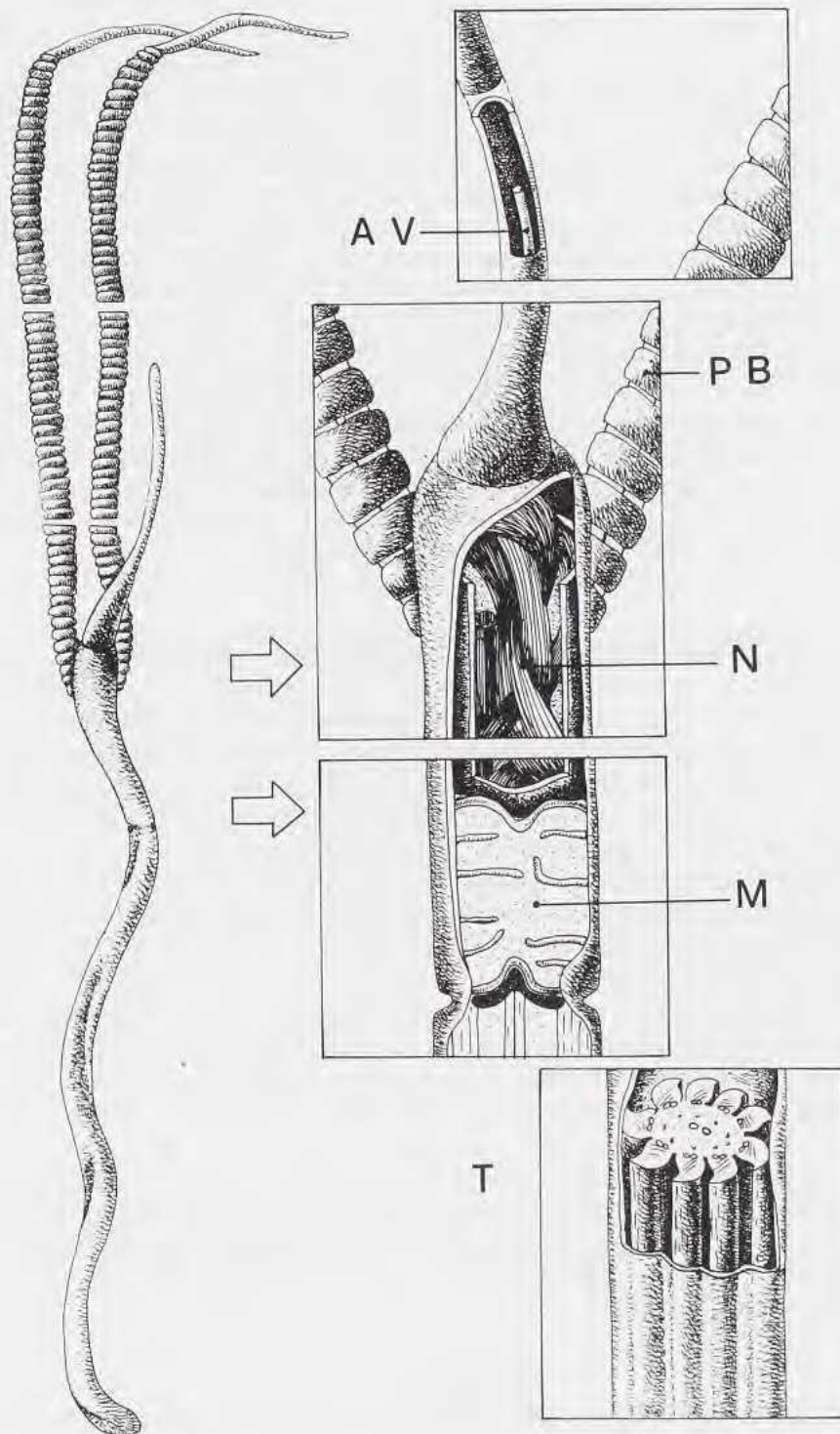


FIG. 6. — Schematic drawing of *Heteroxenotrichula squamosa* spermatozoon. AV, acrosome vesicle; PB, paraacrosomal body; N, nucleus; M, mitochondrion; T, tail.

defined as an "acrosome" in this species would remain to be established. The acrosome of Macrodasysidae briefly described by RUPPERT [10] may share some features with that of the other Macrodasysida. In *Macrodasys* sp., in fact, the acrosome has two portions: the basal one with a "hollow central acrosomal filament" and the anterior one with the filament widening and twisting. A marked difference lies in the mitochondrial spiral winding around the acrosome in *Macrodasys*. The sperm nucleus of all the species, with the exception of *Macrodasys* sp. and, reportedly, of *Thaumastoderma* sp. [11] is a spiral surrounding the cytoplasm. Variations concern the number and morphology of mitochondria and the presence of the additional tubular structure coiled around the nucleus. This additional tubular structure runs along the whole nucleus in some species but is restricted to the nuclear apex in others and may be continuous with the acrosome. In *Cephalodasys maximus*, part of the acrosome is reported to involve the first gyre of the nuclear helix [8]. Mitochondria may retain their conventional aspect, as in *P. etrusca*, or be fused in a single, twisted column-shaped, mitochondrion filling the whole space delimited by the nuclear spiral, as in *Turbanella* (this study and [12, 13]) and *Cephalodasys* [8].

The tail shows a characteristic sheath called the striated cylinder [6], or the spiralled band [8], enclosing the 9+2 axoneme in all families except the Turbanellidae. In *Cephalodasys maximus* in addition to the striated cylinder there are nine dense strings [8] (probably accessory fibres), one corresponding with each flagellar doublet. In this last species, a manchette of oblique microtubules is reported beneath the flagellar plasma membrane.

### *Chaetonotida*

The spermatozoa of Chaetonotida show a much greater interfamilial diversity with respect to Macrodasysida. The sperm models of *Neodasys* [11], Xenotrichulidae ([7] and this chapter), and Chaetonotidae [3, 9] studied so far are different from one another and bear no resemblance to those of Macrodasysida. In particular the spermatozoa of Xenotrichulidae are unique not only among Gastrotricha, but in the entire animal kingdom in the large paraacrosomal bodies (not present, for unknown reasons, in one of the three species examined). The other outstanding characters of the xenotrichulid spermatozoa are: the presence of a single, untransformed mitochondrion placed between the nucleus and the tail and of the large accessory fibres of the flagellum. The shape and position of the single mitochondrion is a curious convergence with leech spermatozoa [14], whereas the accessory fibres are an independent invention of many evolutionary lineages with internal fertilization [2].

Present knowledge of gastrotrich sperm morphology does not allow the delineation of a general sperm model for Gastrotricha. Furthermore, if some characters are common among most Macrodasysida (complex corkscrew-shaped acrosome, spring-shaped nucleus containing mitochondria, striated cylinder in the tail), the three Chaetonotida models known so far present a puzzling diversity.

Comparisons with the spermatozoa of other aschelminthes are also deceiving. The general architecture of gastrotrich spermatozoa seems unique among pseudocoelomates. We may note that if the tubular spiral structure involving the nucleus of some Thaumastodermatidae is proved to be continuous with the acrosome, this could be an important resemblance with the spermatozoon of the priapulid *Tubiluchus corallicola* [1].

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